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Application No.: 10/551,977

Docket No.: KZI-002US

In the claims:*Please amend claims 6 and 12.*

1. **(Previously presented)** A recombinant fusion peptabody, which binds to an epidermal growth factor receptor selected from the group consisting of ErbB-1, ErbB-3, and or ErbB-4, comprising:

- (a) a portion of a cartilage oligomer matrix polypeptide;
- (b) a peptide enhancer sequence for increasing protein production located at the N terminus of the portion of the cartilage oligomer matrix polypeptide;
- (c) a portion of a hinge region of an immunoglobulin polypeptide located at the C terminus of the portion of the cartilage oligomer matrix polypeptide; and
- (d) an epidermal growth factor receptor ligand located at the C terminus of the hinge region,

wherein said recombinant fusion peptabody is capable of inducing cellular death in a cell expressing said epidermal growth factor receptor.

2. **(Canceled)**

3. **(Canceled)**

4. **(Previously presented)** The recombinant fusion peptabody of claim 1, wherein said recombinant fusion peptabody is multimeric.

5. **(Previously presented)** The recombinant fusion peptabody of claim 1, wherein the peptide enhancer sequence is selected from the group consisting of: YSFE, YSFEDL, YSFEDLY, YSFEDLYR, and YSFEDLYRR.

6. **(Currently amended)** The recombinant fusion peptabody of claim 1, ~~claims 1-5~~, wherein said epidermal growth factor receptor ligand is selected from the group consisting of:

- (a) an epidermal growth factor polypeptide or fragments or variants thereof,

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- (b) a growth blocking peptide or fragments or variants thereof,
- (c) a TGF alpha polypeptide or fragments or variants thereof,
- (d) a plasmocyte spreading peptide or fragments or variants thereof,
- (e) a paralytic peptide or fragments or variants thereof,
- (f) a cardioactive peptide or fragments or variants thereof,
- (g) an amphiregulin polypeptide or fragments or variants thereof,
- (h) a heparin-binding epidermal growth factor-like polypeptide or fragments or variants thereof,
- (i) a betacellulin polypeptide or fragments or variants thereof, and or
- (j) a viral EGF-like polypeptide or fragments or variants thereof.

7. **(Previously presented)** The recombinant fusion peptabody of claim 6, wherein said epidermal growth factor receptor ligand is present in its full-length sequences.

8. **(Previously presented)** The recombinant fusion peptabody of claim 1, further comprising a polyhistidine tag sequence.

9. **(Previously presented)** The recombinant fusion peptabody of claim 1, further comprising at least one effector region.

10. **(Previously presented)** The recombinant fusion peptabody of claim 9, wherein the effector region comprises a cytotoxin or a detection moiety.

11. **(Canceled)**

12. **(Currently amended)** The recombinant fusion peptabody of claim 10 ~~11~~, wherein said detection moiety is fluorescent.

13. **(Previously presented)** An isolated nucleic acid comprising a DNA sequence encoding the recombinant fusion peptabody of claim 1.

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14. **(Previously presented)** A vector comprising at least one copy of the isolated nucleic acid of claim 13.

15. **(Previously presented)** The vector of claim 14, further comprising a promoter operably linked to said isolated nucleic acid.

16. **(Previously presented)** A prokaryotic or eukaryotic host cell capable of expressing the isolated nucleic acid of claim 13.

17. **(Previously presented)** A pharmaceutical composition comprising the recombinant fusion peptabody of claim 1, and a pharmaceutically acceptable carrier.

18. **(Canceled)**

19. **(Canceled)**

20. **(Canceled)**

21. **(Previously presented)** A method of treating or preventing cancer characterized by expression of an epidermal growth factor receptors selected from the group consisting of ErbB1, ErbB3, and ErbB4, comprising administering the pharmaceutical composition of claim 17 to a subject, wherein the cancer is selected from the group consisting of carcinoma, lymphoma, blastoma, sarcoma, liposarcoma, neuroendocrine tumor, mesothelioma, schwannoma, meningioma, adenocarcinoma, melanoma, leukemia, lymphoid malignancy, squamous cell cancer, epithelial squamous cell cancer, lung cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic

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carcinoma, anal carcinoma, penile carcinoma, testicular cancer, esophageal cancer, a tumor of the biliary tract, head cancer, and neck cancer.

22. (Canceled)

23. (Previously presented) A method for inducing apoptosis or necrosis, comprising contacting a cell with the recombinant fusion peptabody of claim 1.

24. (Original) The method of claim 23, wherein said cell is a cancer cell.

25. (Previously presented) A method for inhibiting cell proliferation, comprising contacting a cell with the recombinant fusion peptabody of claim 1.

26. (Original) The method of claim 25, wherein said cell is a cancer cell.

27. (Previously presented) A method of diagnosing cancer comprising administering to a subject the recombinant fusion peptabody of claim 10.

28. (Previously presented) A kit for treating cancer characterized by expression of an epidermal growth factor receptors selected from the group consisting of ErbB1, ErbB3, and ErbB4, in a human patient, said kit comprising the recombinant fusion peptabody of claim 1 and/or instructions for administering the recombinant fusion peptabody to the human patient for the treatment of cancer.

29. (Previously presented) The kit of claim 28, further comprising a separate pharmaceutical dosage form comprising an additional anti-cancer agent selected from the group consisting of a chemotherapeutic agents, an anti-epidermal growth factor receptors antibody, a radioimmunotherapeutic agents, and combinations thereof.

30. (Previously presented) A kit for diagnosing cancer characterized by expression of an epidermal growth factor receptors selected from the group consisting of ErbB1, ErbB3, and

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ErbB4, in a human patient, said kit comprising the recombinant fusion peptabody of claim 10, and instructions for use.

31. **(Previously presented)** A method for producing the recombinant fusion peptabody of claim 1, comprising:

- a) constructing a DNA molecule encoding the recombinant fusion peptabody of claim 1;
- b) allowing expression of said DNA molecule in a cell expression system under suitable conditions; and
- c) recovering the recombinant fusion peptabody.

32. **(Original)** The method of claim 31, characterized in that the cell expression system is a prokaryotic cell.

33. **(Previously presented)** The method of claim 31, characterized in that the suitable conditions comprise culturing the cell expression system at a temperature between 10-40 °C during 2-40 hours.

34. **(Previously presented)** The method of claim 33, characterized in that the suitable conditions comprise culturing the cell expression system at a temperature of 37°C during 8-16 hours.

35. **(Previously presented)** The method of claim 31, characterized in that step c) is achieved by extraction of said recombinant fusion peptabody from the cell expression system subsequently followed by purification and refolding steps.

36. **(Original)** The method of claim 35, characterized in that the purification is carried out in the presence of reducing agents and results in the elimination of contamination.

37. **(Original)** The method of claim 35, characterized in that the refolding step is carried out by direct dilution in refolding buffer and further comprises serial dialysis.

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38. **(Previously presented)** The method of claim 37, further characterized by at least one of the following conditions:

- a) the direct dilution in refolding buffer leads to a final concentration of the recombinant fusion peptabody below 300 nM;
- b) the serial dialysis comprise at least 2 different dialysis buffers; or
- c) the refolding step consists in the oxidation of the recombinant fusion peptabody before its concentration.

39. **(Canceled)**

40. **(Canceled)**

41. **(Previously presented)** An isolated peptide enhancer sequence comprising an amino acid sequence selected from the group consisting of: YSFE, YSFEDL, YSFEDLY, YSFEDLYR, YSFEDLYRR, a molecular chimera thereof, and variants thereof.

42. **(Previously presented)** A recombinant protein comprising the enhancer peptide of claim 41.

43. **(Previously presented)** A recombinant fusion peptabody, which binds to the epidermal growth factor receptor ErbB-1 comprising:

- (a) a portion of a cartilage oligomer matrix polypeptide;
- (b) a peptide enhancer sequence for increasing protein production, located at the N terminus of the portion of the cartilage oligomer matrix polypeptide and having a sequence selected from the group consisting of YSFE, YSFEDL, YSFEDLY, YSFEDLYR, and YSFEDLYRR;
- (c) a portion of a hinge region of an immunoglobulin polypeptide located at the C terminus of the portion of the cartilage oligomer matrix polypeptide; and
- (d) an epidermal growth factor receptor ligand located at the C terminus of the hinge region,

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wherein said recombinant fusion peptabody is capable of inducing cellular death in a cell expressing the epidermal growth factor receptor.

44. (Previously presented) A monomer of a peptabody comprising
- (a) a portion of a cartilage oligomer matrix polypeptide;
 - (b) an enhancer peptide sequence located at the N terminus of the portion of the cartilage oligomer matrix polypeptide;
 - (c) a portion of a hinge region of an immunoglobulin polypeptide located at the C terminus of the portion of the cartilage oligomer matrix polypeptide; and
 - (d) an epidermal growth factor receptor ligand located at the C terminus of the hinge region, wherein the epidermal growth factor receptor ligand binds to an epidermal growth factor receptor selected from the group consisting of ErbB-1, ErbB-3 or ErbB-4.
45. (Previously presented) The monomer of claim 44, wherein said monomer forms a multimeric molecule.
46. (Previously presented) The monomer of claim 45, wherein the multimeric molecule is pentameric or decameric.
47. (Previously presented) The monomer of 44, wherein the enhancer peptide sequence is selected from the group consisting of YSFE, YSFEDL, YSFEDLY, YSFEDLYR, YSFEDLYRR, and variants thereof.
48. (Previously presented) An isolated nucleic acid comprising a DNA sequence encoding the monomer of claim 44.
49. (Previously presented) An isolated and recombinant fusion peptabody, which binds to an epidermal growth factor receptor selected from the group consisting of ErbB-1, ErbB-3, and ErbB-4, comprising:
- (a) a portion of a humanized or human cartilage oligomer matrix polypeptide;

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(b) a peptide enhancer sequence for increasing protein production located at the N terminus of the portion of the cartilage oligomer matrix polypeptide;

(c) a portion of a hinge region of an immunoglobulin polypeptide located at the C terminus of the portion of the cartilage oligomer matrix polypeptide; and

(d) an epidermal growth factor receptor ligand located at the C terminus of the hinge region,

wherein said isolated and recombinant fusion peptabody is capable of inducing cellular death in a cell expressing said epidermal growth factor receptor.